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August 27, 1999

ATTN: BOX PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

ATTN: BOX PCT

Re: Application of Hideaki TADA, Mikio KONISHI and Daikichi FUKUSHIMA
NOVEL POLYPEPTIDES, DNAs ENCODING THE POLYPEPTIDES, AND UTILITY OF THE POLYPEPTIDES
Our Reference: Q55589
PCT/JP98/00799, filed February 26, 1998

Dear Sir:

Applicants herewith submit the attached papers for the purpose of entering the National stage under 35 U.S.C. § 371 and in accordance with Chapter II of the Patent Cooperation Treaty. Attached hereto is the application identified above which is a translation of **PCT International Application No. PCT/JP98/00799, filed February 26, 1998**, comprising the specification, claims, one (1) sheet of drawing, executed Declaration and Power of Attorney, foreign language International Preliminary Examination Report, Information Disclosure Statement, PTO Form 1449 with references, International Search Report, executed Assignment and PTO Form 1595.

The Government filing fee is calculated as follows:

Total Claims	11 - 20 =	0 x $18 =	$ 000.00
Independent Claims	1 - 3 =	0 x $78 =	$ 000.00
Base Filing Fee	($840.00)		$ 840.00
Multiple Dep. Claim Fee	($260.00)		$ 260.00
TOTAL FILING FEE			**$ 1100.00**
Recordation of Assignment Fee			$ 40.00
TOTAL U.S. GOVERNMENT FEE			**$ 1140.00**

Checks for the statutory filing fee of **$ 1,100.00** and Assignment recordation fee of **$ 40.00** are attached. You are also directed and authorized to charge or credit any difference or overpayment to Deposit Account No. 19-4880. The Commissioner is hereby authorized to charge any fees under 37 C.F.R. 1.16 and 1.17 which may be required during the entire pendency of the application to Deposit Account No. 19-4880. A duplicate copy of this transmittal letter is attached.

Priority is claimed from:

Japanese Patent Application	Filing Date
P. Hei. 9-43143	February 27, 1997

Respectfully submitted,
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Specification

Novel polypeptides, DNAs encoding the polypeptides, and Utility of the polypeptides

Technical Field

The invention is related to novel polypeptides produced by a certain human stromal cell line and DNAs encoding the said polypeptides.

More particularly, the invention is related to novel polypeptides named to OAF065α and OAF065β (called them OAF065s hereafter), a process for the preparation them, DNAs encoding the said polypeptides, a vector containing the polypeptide, a host cell transformed by the vector, antibody of the said polypeptide, a pharmaceutical composition containing the polypeptide or antibody.

Technical Background

It is known that bone marrow stromal cells form bone marrow micro environment of immunologic, hematopoietic system etc, and they produce and secret essential factors to induce of proliferation and differentiation of stem cells, e.g. IL-7, SCF, IL-11, M-CSF, G-CSF, GM-CSF, IL-6, TGF-β, LIF etc. It is also made clear that a certain bone marrow stromal cells are related to bone metabolism (Kenneth Dorshkind Annu. Rev. Immunol. 8, 111-137. 1990). However, roles of stromal cell are not reconstituted completely from only isolated factors yet. It may suggest that existence of any factors

which are not isolated yet.

Disclosure of the invention

The present inventors have directed their attention to this point and energetic research has been carried out in order to find novel factors (polypeptides) especially secretory and membrane protein which are generated by a certain stromal cells.

Until now, when a man skilled in the art intends to obtain a particular polypeptide or a DNA encoding it, he generally utilizes methods by confirming an intended biological activity in a tissue or in a cell medium, isolating and purifying the polypeptide and then cloning a gene or methods by "expression-cloning" with the guidance of the biological activity.

However, physiologically active polypeptides in living body have often many kinds of activities. Therefore, it is increasing that after a gene is cloned, the gene is found to be identical to that encoding a polypeptide already known. Generally bone marrow stromal cell generates only a very slight amount of a factor and it makes difficult to isolate and to purify the factor and to confirm its biological activity.

Recent rapid developments in techniques for constructing cDNAs and sequencing techniques have made it possible to quickly sequence a large amount of cDNAs. By utilizing these techniques, a process, which comprises constructing cDNAs at random, identifying the nucleotide sequences thereof, expressing novel

polypeptides encoded by them, is now in progress. Although this process is advantageous in that a gene can be cloned and information regarding its nucleotide sequence can be obtained without any biochemical or genetic analysis, the target gene can be discovered thereby only accidentally in many cases.

The present inventors have studied cloning method of genes coding proliferation and/or differentiation factors functioning in hematopoietic systems and immune systems. Focusing their attention on the fact that most of the secretory proteins such as proliferation and/or differentiation factors (for example various cytokines) and membrane proteins such as receptors thereof (hereafter these proteins will be referred to generally as secretory proteins and the like) have sequences called signal peptides in the N-termini, the inventors conducted extensive studies on a process for efficiently and selectively cloning a gene coding for a signal peptide. Finally, we have successfully invented a screening method for cDNAs having sequence encoding signal peptides, we called the method as signal sequence trap (SST) (See Japanese Patent Application No. 6-13951). We also developed yeast SST method on the same concept. By the method using yeast, genes including sequence encoding signal peptide can be identified more easily and effectively (See USP No. 5,536,637).

By using SST method, the present inventors achieved to find novel membrane proteins produced by bone marrow stromal cell and DNAs encoding them, and we then completed the invention.

The polypeptide OAF065s of the invention are not known one, when amino acid sequences of the polypeptide was compared by a computer to all known sequences in data base of Swiss Prot Release 33. It was found out that the polypeptides of the invention are type-I membrane protein and they have extracellular Cys rich region which commonly exists in the receptor family of Tumor necrosis factor (TNF) (See Fig. 1). So it was suggested that the polypeptides of the invention are novel membrane proteins which belong to TNF receptor family.

Brief Description of the Drawing

Fig. 1 shows comparison of the amino acid sequence of the invention and that of TNF receptor family. hTNFR1 represents human necrosis factor receptor 1, hTNFR2 represents human necrosis factor receptor 2, hNGFR represents human nerve growth factor receptor, and hFas represents human Fas, in this figure.

Detailed Description of the invention

The invention provides:

1) a polypeptide comprising an amino acid sequence shown in SEQ ID NO. 1 or NO. 5,

2) a DNA encoding the polypeptides described above (1),

3) a DNA comprising a nucleotide sequence shown in SEQ ID NO. 2 or NO. 6,

4) a DNA comprising a nucleotide sequence shown in SEQ ID NO. 3 or NO. 7.

More particularly, the invention is concerned with a polypeptide comprising amino acid sequence shown in SEQ ID NO. 1 or 5 in substantially purified form, a homologue thereof, a fragment of the sequence and a homologue of the fragment.Further, the invention is concerned with DNAs encoding the above peptides. More particularly the invention is provided DNAs comprising nucleotide sequence shown in SEQ ID NO. 2, 3, 6 or 7, and DNA containing a fragment which is selectively hybridizing to the DNA comprising nucleotide sequence shown in SEQ ID NO. 2, 3, 6 or 7.

A polypeptide comprising amino acid sequence shown in SEQ ID NO. 1 or 5 in substantially purified form will generally comprise the polypeptide in a preparation in which more than 90%, e.g. 95%, 98% or 99% of the polypeptide in the preparation is that of the SEQ ID NO. 1 or 5. A homologue of polypeptide comprising amino acid sequence shown in SEQ ID NO. 1 or 5 will be generally at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the polypeptide comprising amino acid sequence shown in SEQ ID NO. 1 over a region of at least 20, preferably at least 30, for instance 40, 60 or 100 more contiguous amino acids. Such a polypeptide homologue will be referred to a polypeptide of the invention.

Generally, a fragment of polypeptide comprising amino acid sequence shown in SEQ ID NO. 1 or 5 or its homologues will be at

least 10, preferably at least 15, for example 20, 25, 30, 40, 50 or 60 amino acids in length, and are also referred to by the term "a polypeptide of the invention".

A DNA capable of selectively hybridizing to the DNA comprising nucleotide sequence shown in SEQ ID NO. 2, 3, 6 or 7 will be generally at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the DNA comprising nucleotide sequence shown in SEQ ID NO. 2, 3, 6 or 7 over a region of at least 20, preferably at least 30, for instance 40, 60 or 100 or more contiguous nucleotides. Such DNA will be referred to "a cDNA of the invention".

Fragments of the DNA comprising nucleotide sequence shown in SEQ ID NO. 2, 3, 6 or 7 will be at least 10, preferably at least 15, for example 20, 25, 30 or 40 nucleotides in length, and will be also referred to "a DNA of the invention" as used herein.

A further embodiment of the invention provides replication and expression vectors carrying DNA of the invention. The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said DNA and optionally a regulator of the promoter. The vector may contain one or more selectable marker genes, for example a ampicillin resistance gene. The vector may be used in vitro, for example of the production of RNA corresponding to the cDNA, or used to transfect or transfect a host cell.

A further embodiment of the invention provides host cells

transformed with the vectors for the replication and expression of the DNA of the invention, including the DNA SEQ ID NO. 2, 3, 6 or 7 or the open reading frame thereof. The cells will be chosen to be compatible with the vector and may for example be bacterial, yeast, insect or mammalian.

A further embodiment of the invention provides a method of producing a polypeptide which comprises culturing host cells of the invention under conditions effective to express a polypeptide of the invention. Preferably, in addition, such a method is carried out under conditions in which the polypeptide of the invention is expressed and then produced from the host cells.

DNA of the invention may also be inserted into the vectors described above in an antisense orientation in order to proved for the production of antisense RNA. Such antisense RNA may be used in a method of controlling the levels of a polypeptide of the invention in a cell.

The invention also provides monoclonal or polyclonal antibodies against a polypeptide of the invention. The invention further provides a process for the production of monoclonal or polyclonal antibodies to the polypeptides of the invention. Monoclonal antibodies may be prepared by common hybridoma technology using polypeptides of the invention or fragments thereof, as an immunogen. Polyclonal antibodies may also be prepared by common means which comprise inoculating host animals, for example a rat or a rabbit, with polypeptides of the invention and recovering

immune serum.

The invention also provides pharmaceutical compositions containing a polypeptide of the invention, or an antibody thereof, in association with a pharmaceutically acceptable diluent and/or carrier.

The polypeptide of the invention includes that which a part of their amino acid sequence is lacking (e.g., a polypeptide comprised of the only essential sequence for revealing a biological activity in an amino acid sequence shown in SEQ ID NO.1), that which a part of their amino acid sequence is replaced by other amino acids (e.g., those replaced by an amino acid having a similar property) and that which other amino acids are added or inserted into a part of their amino acid sequence, as well as those comprising the amino acid sequence shown in SEQ ID NO. 1 or 5.

As known well, there are one to six kinds of codon as that encoding one amino acid (for example, one kind of codon for Methioine (Met), and six kinds of codon for leucine (Leu) are known). Accordingly, the nucleotide sequence of DNA can be changed in order to encode the polypeptide having the same amino acid sequence.

The DNA of the invention, specified in (2) includes a group of every nucleotide sequences encoding polypeptides (1) shown in SEQ ID NO. 1 or 5. There is a probability that yield of a polypeptide is improved by changing a nucleotide sequence.

The DNA specified in (3) is the embodiment of the DNA shown in (2), and indicate the sequence of natural form.

The DNA shown in (4) indicates the sequence of the DNA specified in (3) with natural non-translational region.

cDNA carrying nucleotide sequence shown in SEQ ID NO. 3 is prepared by the following method:

Brief description of Yeast SST method (see USP No. 5,536,637) is as follows.

Yeast such as Saccharomyces cerevisiae should secrete invertase into the medium in order to take sucrose or raffinose as a source of energy or carbon (Invertase is an enzyme to cleave raffinose into sucrose and melibiose, sucrose into fructose and glucose.). It is known that many known mammalian signal sequence make yeast secrete its invertase. From these knowledge, SST method was developed as a screening method to find novel signal sequence which make it possible can to secrete yeast invertase from mammalian cDNA library. SST method uses yeast growth on raffinose medium as a marker. Non-secretory type invertase gene SUC2 (GENBANK Accession No. V 01311) lacking initiation codon ATG was inserted to yeast expression vector to prepare yeast SST vector pSUC2. In this expression vector, ADH promoter, ADH terminator (both were derived from AAH5 plasmid (Gammerer, Methods in Enzymol. 101, 192-201, 1983)), 2μ ori (as a yeast replication origin), TRP1 (as a yeast selective marker), ColE1 ori(as a E. Coli replication origin) and ampicillin resistance gene (as a drug resistance marker) were inserted. Mammalian cDNA was inserted into the upstream of SUC2 gene to prepare yeast SST cDNA library. Yeast

lacking secretory type invertase, was transformed with this library. If inserted mammalian cDNA encodes a signal peptide, yeast could be survive in raffinose medium as a result of restoring secretion of invertase. Only to culture yeast colonies, prepare plasmids and determine the nucleotide sequence of the insert cDNAs, it is possible to identify novel signal peptide rapidly and easily.

Preparation of yeast SST cDNA library is as follows:

(1) mRNA is isolated from the targeted cells, second-strand synthesis is performed by using random primer with certain restriction enzyme (enzyme I) recognition site,

(2) double-strand cDNA is ligated to adapter containing certain restriction endonuclease (enzyme II) recognition site, differ from enzyme I, digested with enzyme I and fractionated in a appropriate size,

(3) obtained cDNA fragment is inserted into yeast expression vector on the upstream region of invertase gene which signal peptide is deleted and the library was transformed.

Detailed description of each step is as follows:

(1) mRNA is isolated from mammalian organs and cell lines stimulate them with appropriate stimulator if necessary) by known methods (Molecular Cloning (Sambrook, J., Fritsch, E. F. and Maniatis, T., Cold Spring Harbor Laboratory Press, 1989) or Current Protocol in Molecular Biology (F. M. Ausubel et al, John Wiley & Sons, Inc.)

if not remark especially).

HAS303 (human bone marrow stromal cell line: provide from Professor Keisuke Sotoyama, Dr. Makoto Aizawa of Tokyo Medical College, 1st medicine; see J. Cell. Physiol., 148, 245-251, 1991 and Experimental Hematol., 22, 482-487, 1994) and HUVEC (human umbilical vein cord endothelial cell: ATCC No. CRL-1730) are chosen as a tissue source. Double-strand cDNA synthesis using random primer is performed by known methods.

Any sites may be used as restriction endonuclease recognition site I which is linked to adapter and restriction endonuclease recognition site II which is used in step (2), if both sites are different each other. Preferably, EcoRI is used as enzyme I and XhoI as enzyme II.

In step (2), cDNA is created blunt-ends with T4 DNA polymerase, ligated enzyme II adapter and digested with enzyme I. Fragment cDNA is analyzed with agarose-gel electrophoresis (AGE) and is selected cDNA fraction ranging in size from 300 to 800 bp. As mentioned above, any enzyme may be used as enzyme II if it is not same the enzyme I.

In step (3), cDNA fragment obtained in step (2) is inserted into yeast expression vector on the upstream region of invertase gene which signal peptide is deleted. E. coli transformed with the expression vector. Many vectors are known as yeast expression plasmid vector. For example, YEp24 is also functioned in E. Coli. Preferably pSUC2 as described above is used.

Many host E. Coli strains are known for transformation, preferably DH10B competent cell is used. Any known transformation method is available, preferably it is performed by electropolation method. Transformant is cultured by conventional methods to obtain cDNA library for yeast SST method.

However not every All of the clones do not contain cDNA fragment. Further all of the gene fragments do not encode unknown signal peptides. It is therefore necessary to screen a gene fragment encoding for an unknown signal peptide from the library.

Therefore, screening of fragments containing a sequence encoding an appropriate signal peptide is performed by transformation of the cDNA library into Saccharomyces cerevisiae (e.g. YT455 strain) which lack invertase (it may be prepared by known methods.). Transformation of yeast is performed by known methods, e.g. lithium acetate method. Transformant is cultured in a selective medium, then transferred to a medium containing raffinose as a carbon source. Survival colonies are selected and then prepared plasmid. Survival colonies on a raffinose-medium indicates that some signal peptide of secretory protein was inserted to this clone.

Isolated positive clones is determined the nucleotide sequence. As to a cDNA encodes unknown protein, full-length clone may be isolated by using cDNA fragment as a probe and then determined to obtain full-length nucleotide sequence. These manipulation is performed by known methods.

Once the nucleotide sequences shown in SEQ ID NO. 2, 3, 6 or 7 are determined partially or preferably fully, it is possible to obtain DNA encode mammalian protein itself, homologue or subset. cDNA library or mRNA derived from mammals was screened by PCR with any synthesized oligonucleotide primers or by hybridization with any fragment as a probe. It is possible to obtain DNA encodes other mammalian homologue protein from other mammalian cDNA or genome library.

If a cDNA obtained above contains a nucleotide sequence of cDNA fragment obtained by SST (or consensus sequence thereof), it will be thought that the cDNA encodes signal peptide. So it is clear that the cDNA will be full-length or almost full.
(All signal sequences exist at N-termini of a protein and are encoded at 5'-temini of open reading frame of cDNA.)

The confirmation may be carried out by Northern analysis with the said cDNA as a probe. It is thought that the cDNA is almost complete length, if length of the cDNA is almost the same length of the mRNA obtained in the hybridizing band.

Once the nucleotide sequences shown in SEQ ID NOs. 2, 3, 6 or 7 are determined, DNAs of the invention are obtained by chemical synthesis, or by hybridization making use of nucleotide fragments which are chemically synthesized as a probe. Furthermore, DNAs of the invention are obtained in desired amount by transforming a vector that contains the DNA into a proper host, and culturing the transformant.

The polypeptides of the invention may be prepared by:

(1) isolating and purifying from an organism or a cultured cell,

(2) chemically synthesizing, or

(3) using recombinant DNA technology,

preferably, by the method described in (3) in an industrial production.

Examples of expression system (host-vector system) for producing a polypeptide by using recombinant DNA technology are the expression systems of bacteria, yeast, insect cells and mammalian cells.

In the expression of the polypeptide, for example, in E. Coli, the expression vector is prepared by adding the initiation codon (ATG) to 5' end of a DNA encoding mature peptide, connecting the DNA thus obtained to the downstream of a proper promoter (e.g., trp promoter, lac promoter, λ PL promoter, T7 promoter etc.), and then inserting it into a vector (e.g., pBR322, pUC18, pUC19 etc.) which functions in an E. coli strain.

Then, an E. coli strain (e.g., E. coli DH1 strain, E. coli JM109 strain, E. coli HB101 strain, etc.) which is transformed with the expression vector described above may be cultured in a appropriate medium to obtain the desired polypeptide. When a signal peptide of bacteria (e.g., signal peptide of pel B) is utilized, the desired polypeptide may be also released in periplasm. Furthermore, a fusion protein with other polypeptide may be also produced easily.

In the expression of the polypeptide, for example, in a mammalian cells, for example, the expression vector is prepared by inserting the DNA encoding nucleotide shown in SEQ ID NO. 3 or 7 into the downstream of a proper promoter (e.g., SV40 promoter, LTR promoter, metallothionein promoter etc.) in a proper vector (e.g., retrovirus vector, papilloma virus vector, vaccinia virus vector, SV40 vector, etc.). A proper mammalian cell (e.g., monkey COS-7 cell, Chinese hamster CHO cell, mouse L cell etc.) is transformed with the expression vector thus obtained, and then the transformant is cultured in a proper medium to get a desired polypeptide on the cell membrane. A vector described above can be inserted with deletion mutant DNA that encodes sequence, which is deleted transmembrane region from SEQ ID NOs. 3 or 7 and the expression vector can be transfected into an appropriate mammalian cell. The aimed soluble protein can be secreted into the culture medium. The polypeptide available by the way described above can be isolated and purified by conventional biochemical method.

Industrial Applicability

The polypeptide OAF065s of the invention show significant homology with a series of proteins which belong to TNF receptor family. Proteins, which belong to TNF receptor family, are type-1 membrane protein which have 3 to 6 repeated structure containing 6 Cys residues in the extracellular domain. It has been apparent that the proteins are related to proliferation, differentiation

cell death of various cells by the interaction with ligand thereof (Craig A. Smith et. al., Cell, 76, 959-962, 1994) . For instance, Neuronal growth factor (NGF) receptor / NGF are essential for keeping several kinds of neuronal cells surviving, allowing neuronal tubes to elongate and promoting to make neuronal transmitters (Chao M.V., J. Neurobiol., 25, 1373-1385, 1994) . Fas/FasL is essential for maintaining homeostasis in vivo, such as destruction of cancer cells and removal of auto-reactive lymphocytes via its apoptosis-inducing activity, and also relates to CD4-positive T cell reduction in AIDS, fulminant hepatitis, graft versus host disease (GVHD) after transplantation and the onset of various autoimmune diseases (Nagata S. et. al., Science, 267, 1449-1456, 1995). CD40/CD40L is essential for activating B cells (acceleration of growth and antibody production) via T/B cell interaction (Banchereau J. et. al., Annu. Rev. Immunol., 12, 881-922, 1994). TNF receptor/TNF and lymphotoxin (LT) receptor/LT have activities, such as growth, activation and differentiation induction of various immune and hematopoietic cells, cytotoxicity and growth inhibition of tumor cells, growth and activation of various connective tissues (e.g., endothelial cells, fibroblasts, osteoblasts, etc.) and viral growth inhibition, and are also essential for the morphology or organ formation of lymphoid tissue (Ware C.F. et al., Curr. Topics Microbiol. Immunol., 198, 175-218, 1995).

Since repetitive structures of Cys are present at three points in the extracellular domain of the polypeptide of the invention, it is obvious that this is a novel protein belonging to the TNF receptor family and exerts its activity via a ligand belonging to a known or unknown TNF family. In consequence, it is considered that the polypeptide of the invention and a cDNA molecule which encodes the polypeptide will show one or more of the effects or biological activities (including those which relates to the assays cited below) concerning differentiation, proliferation, growth, survival or cell death of hematopoietic, immune and nerve system cells, immune system functions, proliferation and growth of tumor, inflammations, bone metabolism, etc. The effects or biological activities described in relation to the polypeptide of the invention are provided by administration or use of the polypeptide or by administration or use of a cDNA molecule which encodes the polypeptide (e.g., vector suitable for gene therapy or cDNA introduction).

1) Cytokine activity and cell proliferation/differentiation activity

The polypeptide of the invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a polypeptide of the invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines.

2) Immune stimulating/suppressing activity

The polypeptide of the invention may also exhibit immune stimulating or immune suppressing activity. The polypeptide of the invention may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g. HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases

causes by viral, bacterial, fungal or other infection may be treatable using the polypeptide of the invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, leshmania, malaria and various fungal infections such as candida. Of course, in this regard, a polypeptide of the invention may also be useful where a boost to the immune system generally would be indicated, i.e., in the treatment of cancer.

Such a polypeptide of the invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma or other respiratory problems.

The polypeptide of the invention may also suppress chronic or acute inflammation, such as, for example, that associated with infection (such as septic shock or systemic inflammatory response syndrome (SIRS)), inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-I (such as the effect demonstrated by IL- 11).

3) Hematopoiesis regulating activity

The polypeptide of the invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis.

The said biological activities are concerned with the following all or some example(s). e.g. in supporting the growth

and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility. for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells;

in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression;

in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions;

and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vivo or ex-vivo (i.e. in conjunction with bone marrow transplantation) as normal cells or genetically manipulated for gene therapy.

The activity of the polypeptide of the invention may, among

other means. be measured by the following methods :

4) Tissue generation/regeneration activity

The polypeptide of the invention also may have utility in compositions used for bone, cartilage, tendon, Ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair, and in the treatment of bums, incisions and ulcers. The polypeptide of the invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing the polypeptide of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

The polypeptide of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. The polypeptide of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of

bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the polypeptide of the invention is tendon/ligament formation. A polypeptide of the invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/Ligament-like tissue inducing polypeptide may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon Ligament cells or progenitors ex vivo for return in vivo to effect tissue repair. The compositions of the invention may also

be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The polypeptide of the invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue. i.e. for the treatment of central and peripheral nervous system diseases and neuropathies. as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, the polypeptide of the invention may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using the polypeptide of the invention.

It is expected that the polypeptide of the invention may also exhibit activity for generation of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular

(including vascular endothelium) tissue, or for promoting the proliferation of cells comprising such tissues. Part of the desired effects may be by inhibition of fibrotic scarring to allow normal tissue to regenerate.

A polypeptide of the invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

5) Activin/Inhibin activity

The polypeptide of the invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a polypeptide of the invention alone or in heterodimers with a member of the inhibin α family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the polypeptide of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin-β group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See for example, USP

4,798,885. The polypeptide of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

6) Chemotactic/chemokinetic activity

A polypeptide of the invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including. for example. monocytes, neutrophils, T-cells, mast cells, eosinophils and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilized or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

7) Hemostatic and thrombolytic activity

The polypeptide of the invention may also exhibit hemostatic or thrombolyic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as. for example, infarction or stroke).

8) Receptor/ligand activity

The polypeptide of the invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand

interaction. A polypeptide of the invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

9) Other activity

The polypeptide of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, bacteria, viruses, fungi and other parasites;

effecting (suppressing or enhancing) bodily characteristics, including, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution);

effecting elimination of dietary fat, protein, carbohydrate;

effecting behavioral characteristics, including appetite, libido, stress, cognition (including cognitive disorders), depression and violent behaviors;

providing analgesic effects or other pain reducing effects;

promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages;

in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases.

The polypeptide with above activities, is suspected to have following functions by itself or interaction with its ligands or receptors or association with other molecules. For example,

proliferation or cell death of B cells, T cells and/or mast cells or class specific induction of B cells by promotion of class switch of immunoglobulin genes; differentiation of B cells to antibody-forming cells; proliferation, differentiation, or cell death of precursors of granulocytes; proliferation, differentiation, or cell death of precursors of monocytes-macrophages;

proliferation, of up regulation or cell death of neutrophils, monocytes-macrophages, eosinophils and/or basophils;

proliferation, or cell death of precursors of megakaryocytes; proliferation, differentiation, or cell death of precursors of neutrophils; proliferation, differentiation, or cell death of precursors of T cells and B cells; promotion of production of erythrocytes; sustainment of proliferation of erythrocytes, neutrophils, eosinophils, basophils, monocytes-macrophages, mast cells, precursors of megakaryocyte ; promotion of migration of neutrophils, monocytes-macrophages, B cells and/or T cells; proliferation or cell death of thymocytes; suppression of differentiation of adipocytes; proliferation or cell death of natural killer cells;

proliferation or cell death of hematopoietic stem cells; suppression of proliferation of stem cells and each hematopoietic precursor cells; promotion of differentiation from mesenchymal stem cells to osteoblasts or chondrocytes, proliferation or cell death of mesenchymal stem cells, osteoblasts or chondrocytes and

promotion of bone absorption by activation of osteoclasts and promotion of differentiation from monocytes to osteoclasts.

This peptide is also suspected to function to nervous system, so expected to have functions below; differentiation to kinds of neurotransmitter-responsive neurons, survival or cell death of these cells; promotion of proliferation or cell death of glial cells; spread of neural dendrites; survival or cell death of gangriocytes; proliferation, promotion of differentiation, or cell death of astrocytes; proliferation or survival of peripheral neurons; proliferation or cell death of Schwann cells; proliferation, survival or cell death of motoneurons.

Furthermore, in the process of development of early embryonic, this polypeptide is expected to promote or inhibit the organogenesis of epidermis, brain, backbone, and nervous system by induction of ectoderm, that of notochord connective tissues(bone, muscle, tendon), hemocytes, heart, kidney, and genital organs by induction of mesoderm, and that of digestive apparatus (stomach, intestine, liver, pancreas), respiratory apparatus (lung, trachea) by induction of endoderm. In adult, also, this polypeptide is thought to proliferate or inhibit the above organs.

Therefore, this polypeptide itself is expected to be used as an agent for the prevention or treatment of disease of progression or suppression of immune, nervous, or bone metabolic function, hypoplasia or overgrowth of hematopoietic cells: inflammatory

disease (rheumatism, ulcerative colitis, etc.), decrease of hematopoietic stem cells after bone marrow transplantation, decrease of leukocytes, platelets, B-cells, or T-cells after radiation exposure or chemotherapeutic dosage against cancer or leukemia, anemia, infectious disease, cancer, leukemia, AIDS, bone metabolic disease(osteoporosis etc.), various degenerative disease (Alzheimer's disease, multiple sclerosis, etc.), or nervous lesion.

In addition, since this polypeptide is thought to induce the differentiation or growth of organs derived from ectoderm, mesoderm, and endoderm, this polypeptide is expected to be an agent for tissue repair (epidermis, bone, muscle, tendon, heart, kidney, stomach, intestine, liver, pancreas, lung, and trachea, etc.).

Quantitation of the polypeptide of the invention in the body can be performed using polyclonal or monoclonal antibodies against the polypeptide of the invention. It can be used the study of relationship between this polypeptide and disease or diagnosis of disease, and so on. Polyclonal and monoclonal antibodies can be prepared using this polypeptide or its fragment as an antigen by conventional methods.

Identification, purification or molecular cloning of known or unknown proteins which bind the polypeptide of the invention (preferably polypeptide of extracellular domain) can be performed using the polypeptide of the invention by, for example, preparation of the affinity-column.

Identification of the downstream signal transmission molecules which interact with the polypeptide of the invention in cytoplasma and molecular cloning of the gene can be performed: by west-western method using the polypeptide of the invention (preferably polypeptide of transmembrane region or intracellular domain) or

by yeast two-hybrid system using the cDNA (preferably cDNA encoding transmembrane region or cytoplasmic domain of the polypeptide).

Agonists/antagonists of this receptor polypeptide and inhibitors between receptor and signal transduction molecules can be screened using the polypeptide of the invention.

cDNAs of the invention are useful not only the important and essential template for the production of the polypeptide of the invention which is expected to be largely useful, but also be useful for diagnosis or therapy (for example, treatment of gene lacking, treatment to stop the expression of the polypeptide by antisense DNA (RNA)). Genomic DNA may be isolated with the cDNA of the invention, as a probe. As the same manner, a human gene encoding which can be highly homologous to the cDNA of the invention, that is, which encodes a polypeptide highly homologous to the polypeptide of the invention and a gene of animals excluding mouse which can be highly homologous to the cDNA of the invention, also may be isolated.

[Application to medicaments]

The polypeptide of the invention or the antibody specific for the polypeptide of the invention is administered systemically or topically and in general orally or parenterally for preventing or treating diseases related to incomplete growth or abnormal growth of hematopoietic system cells, acceleration or reduction of nerve system functions or acceleration or reduction of immune system functions, such as inflammatory diseases (e.g., rheumatoid, ulcerative colitis, etc.), cytopenia of hematopoietic stem cells after bone marrow transplantation, cytopenia of leukocytes, platelets, B cells or T cells after radiation treatment or after administration of a chemotherapeutic agent, anemia, infectious diseases, cancer, leukemia, AIDS, and various degenerative diseases (e.g., Alzheimer's disease, multiple sclerosis, etc.), or nerve damage, for preventing or treating metabolic disorder of bones (e.g., osteoporosis, etc.), or for repairing tissues. Oral administration, intravenous injection and intraventricular administration are preferred.

The doses to be administered depend upon age, body weight, symptom, desired therapeutic effect, route of administration, and duration of the treatment etc. In human adults, one dose per person is generally between 100 μg and 100 mg, by oral administration, up to several times per day, and between 10 μg and 100 mg, by parenteral administration up to several times per day.

As mentioned above, the doses to be used depend upon various conditions. Therefore, there are cases in which doses lower than or greater than the ranges specified above may be used.

The compounds of the invention, may be administered as solid compositions, liquid compositions or other compositions for oral administration, as injections, liniments or suppositories etc. for parenteral administration.

Solid compositions for oral administration include compressed tablets, pills, capsules, dispersible powders, granules. Capsules include soft or hard capsules.

In such compositions, one or more of the active compound(s) is or are admixed with at least one inert diluent (such as lactose, mannitol, glucose, hydroxypropyl cellulose, microcrystalline cellulose, starch, polyvinylpyrrolidone, magnesium metasilicate aluminate, etc.). The compositions may also comprise, as is normal practice, additional substances other than inert diluents: e.g. lubricating agents (such as magnesium stearate etc.),

disintegrating agents (such as cellulose calcium glycolate, etc.), stabilizing agents (such as human serum albumin, lactose etc.), and assisting agents for dissolving (such as arginine, asparaginic acid etc.).

The tablets or pills may, if desired, be coated with a film of gastric or enteric materials (such as sugar, gelatin, hydroxypropyl cellulose or hydroxypropylmethyl cellulose phthalate, etc.), or be coated with more than two films. And then, coating may include containment within capsules of absorbable materials such as gelatin.

Liquid compositions for oral administration include pharmaceutically-acceptable emulsions, solutions, syrups and elixirs. In such compositions, one or more of the active compound(s) is or are contained in inert diluent(s) commonly used (purified water, ethanol etc.). Besides inert diluents, such compositions may also comprise adjuvants (such as wetting agents, suspending agents, etc.), sweetening agents, flavoring agents, perfuming agents, and preserving agents.

Other compositions for oral administration include spray compositions which may be prepared by known methods and which comprise one or more of the active compound(s). Spray compositions may comprise additional substances other than inert diluents: e.g. stabilizing agents (sodium sulfite etc.), isotonic buffer (sodium chloride, sodium citrate, citric acid, etc.). For preparation of such spray compositions, for example, the method described in the

United States Patent No. 2,868,691 or 3,095,355 (herein incorporated in their entireties by reference) may be used.

Injections for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions and emulsions. In such compositions, one or more active compound(s) is or are admixed with at least one inert aqueous diluent(s) (distilled water for injection, physiological salt solution, etc.) or inert non-aqueous diluents(s)(propylene glycol, polyethylene glycol, olive oil, ethanol, POLYSOLBATE 80 TM , etc.).

Injections may comprise additional compound other than inert diluents: e.g. preserving agents, wetting agents, emulsifying agents, dispersing agents, stabilizing agent (such as human serum albumin, lactose, etc.), and assisting agents such as assisting agents for dissolving (arginine, asparaginic acid, etc.).

Best Mode carring out the Invention

The invention are illustrated by the following examples, but not limit the invention.

Example

Total RNA was prepared from human bone marrow stromal cell line HAS303 (provided from Professor Keisuke Sotoyama, Dr. Makoto Aizawa, first medicine, Tokyo Medical College; See J. Cell. Physiol., 148 : 245-251 (1991) and Experimental Hematol., 22 : 482-487(1994)) by TRIzol reagent (Trade Mark, GIBCOBRL). Poly(A)RNA was purified from the total RNA by mRNA purification kit (commercial name,

Pharmacia).

Double strand cDNA was synthesized by SuperScript Plasmid System for cDNA Synthesis and Plasmid Cloning (brand name, GIBCOBRL) with above poly(A)RNA as template and random 9mer as primer which was containing XhoI site:

SEQ ID NO. 9

5'-CGA TTG AAT TCT AGA CCT GCC TCG AGN NNN NNN NN-3'

cDNA was ligated EcoRI adapter by DNA ligation kit ver.2 (trade name, Takara Shuzo; this kit was used in all ligating steps hereafter.) and digested by XhoI. cDNAs were separated by agarose-gel electrophoresis. 300 - 800 bp cDNAs were isolated and were ligated to EcoRI/NotI site of pSUC2 (see US 5,536,637). E. Coli DH10B strain were transformed by pSUC2 with electropolation to obtain yeast SST cDNA library.

Plasmids of the cDNA library were prepared. Yeast YTK12 strain were transformed by the plasmids with lithium acetate method (Current Protocols In Molecular Biology 13.7.1). The transformed yeast were plated on triptphan-free medium (CMD-Try medium) for selection. The plate was incubated for 48 hour at 30 °C. Replica of the colony which is obtained by Accutran Replica Plater (trade name, Schleicher & Schuell) were place YPR plate containing raffinose for carbon source, and the plate was incubated for 14 days at 30 °C. After 3 days, each colony appeared was streaked on YPR plate again. The plates were incubated for 48 hours at 30 °C.

Single colony was inoculated to YPR medium and was incubated for 48 hours at 30 °C. Then plasmids were prepared. Insert cDNA was amplified by PCR with two kind primers which exist end side of cloning site on pSUC2 (sense strand primers were biotinylated). Biotinylated single strand of cDNAs were purified with Dynabeads (trade name, DYNAL) and determined the nucleotide sequences. Sequencing was performed by Dye Terminator Cycle Sequencing Ready Reaction with DNA Sequencing kit (trade name, Applied Biosystems Inc.) and sequence was determined by DNA sequencer 373 (Applied Biosystems Inc.). All sequencing hereafter was carried with this method.

The clone named OAF065 is not registered on databases by homology search of nucleotide sequence and deduced amino acid sequence and so it is cleared that the sequence is novel one. We confirmed that OAF065 contains signal peptide in view of function and structure, by comparison with known peptide which has signal peptide and deduced amino acid sequence. Full length cDNA of OAF065 was isolated by 3'-RACE(Rapid Amplification of cDNA End). Marathon cDNA Amplification Kit(trade name, Clontech) was used in 3'-RACE. Adaptor-ligated double stranded cDNA was prepared from poly(A)RNA of HAS303 in line with the method of the kit. OAF065 specific primer F3 (28mer):

SEQ ID NO. 10

5'-AGA AAG ATG GCT TTA AAA GTG CTA CTA G-3'

which included a deduced initiation ATG coden region based on the information of nucleotide sequence by SST was prepared. PCR was performed with the said primer and adapter primer attached in the kit. Two kinds of cDNAs (4.0 kb and 1.5 kb) were amlified and 4.0 kb-cDNA was named OAF065α and 1.5 kb-cDNA was named OAF065β.

Two kinds cDNAs were separated with agarose-gel electrophoresis, and to pT7 Blue-2 T-Vector (trade name, Novagen), ligated in and transformed to E. Coli DH5α and then plasmid was prepared. Nucleotide sequences of 5'-end were determined, and the existance of nucleotide sequence OAF065 specific primer F3 were confirmed in both nucleotide sequences. 5'-End nucleotide sequence (ca 1.7 kb) of OAF065α and full length nucleotide sequence of OAF065β were determined and then obtained sequences shown in SEQ ID NOs 3 and 7. Open reading frame was searched and deduced amino acid sequences shown in SEQ ID NO. 1 and 5 were obtained.

Compared with the nucleotide sequences of OAF065α and OAF065β, nucletide sequences from 1 to 1290 base were completely same, but sequences downstream from 1291 base had no homology each other. Compared with amino acid sequences of OAF065α and OAF065β, amino acids from 1 to 415 in N-termini were completely same, only two amino acids in C-termini of OAF065α were replaced to 8 amino acids (Val Arg Gln Arg Leu Gly Ser Leu) in the sequence of OAF065β. It was revealed that OAF065α and OAF065β were novel type-I membrane proteins by hydrophobisity analysis and that the extracellular region and the transmembrane region of both sequences were

consistant.

The polypeptide OAF065α and OAF065β of the invention are not known one, when amino acid sequences of the polypeptide was compared by a computer to all known sequences in data base of Swiss Prot Release 33. Extracellular Cys rich region which commonly exists in the TNF receptor family was identfied in the polypeptide of the invention.

That is, compared with amino acid sequences of the polypeptide of the invention (OAF065s) and other members of TNF receptor family i.e. human necrosis factor receptor 1 (hTNFR1), human necrosis factor receptor 2 (hTNFR2), human nerve growth factor receptor (hNGFR), and human Fas (hFas), it was revealed that the polypeptides (OAF065s) of the invention are type-I membrane protein and they have extracellular Cys rich region which commonly exists in the TNF (Tumor necrosis factor) receptor family in Fig. 1.

Therefore, it was confirmed that the polypeptides OAF065α and OAF065β of the invention are novel membrane proteins which belong to the TNF receptor family.

SEQUENCE LIST

SEQ ID NO.: 1

Length: 417 amino acid

Type: amino acid

Topology: linear

Molecule type: protein

Sequence

```
Met Ala Leu Lys Val Leu Leu Glu Gln Glu Lys Thr Phe Phe Thr Leu
 1               5                  10                  15
Leu Val Leu Leu Gly Tyr Leu Ser Cys Lys Val Thr Cys Glu Thr Gly
            20                  25                  30
Asp Cys Arg Gln Gln Glu Phe Arg Asp Arg Ser Gly Asn Cys Val Pro
        35                  40                  45
Cys Asn Gln Cys Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe
    50                  55                  60
Gly Tyr Gly Glu Asp Ala Gln Cys Val Thr Cys Arg Leu His Arg Phe
65                  70                  75                  80
Lys Glu Asp Trp Gly Phe Gln Lys Cys Lys Pro Cys Leu Asp Cys Ala
                85                  90                  95
Val Val Asn Arg Phe Gln Lys Ala Asn Cys Ser Ala Thr Ser Asp Ala
            100                 105                 110
Ile Cys Gly Asp Cys Leu Pro Gly Phe Tyr Arg Lys Thr Lys Leu Val
        115                 120                 125
Gly Phe Gln Asp Met Glu Cys Val Pro Cys Gly Asp Pro Pro Pro Pro
    130                 135                 140
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Tyr Glu Pro His Cys Ala Ser Lys Val Asn Leu Val Lys Ile Ala Ser
145 150 155 160
Thr Ala Ser Ser Pro Arg Asp Thr Ala Leu Ala Ala Val Ile Cys Ser
165 170 175
Ala Leu Ala Thr Val Leu Leu Ala Leu Leu Ile Leu Cys Val Ile Tyr
180 185 190
Cys Lys Arg Gln Phe Met Glu Lys Lys Pro Ser Trp Ser Leu Arg Ser
195 200 205
Gln Asp Ile Gln Tyr Asn Gly Ser Glu Leu Ser Cys Leu Asp Pro Arg
210 215 220
Gln Leu His Glu Tyr Ala His Arg Ala Cys Cys Gln Cys Arg Arg Asp
225 230 235 240
Ser Val Gln Thr Cys Gly Pro Val Arg Leu Leu Pro Ser Met Cys Cys
245 250 255
Glu Glu Ala Cys Ser Pro Asn Pro Ala Thr Leu Gly Cys Gly Val His
260 265 270
Ser Ala Ala Ser Leu Gln Ala Arg Asn Ala Gly Pro Ala Gly Glu Met
275 280 285
Val Pro Thr Phe Phe Gly Ser Leu Thr Gln Ser Ile Cys Gly Glu Phe
290 295 300
Ser Asp Ala Trp Pro Leu Met Gln Asn Pro Met Gly Gly Asp Asn Ile
305 310 315 320
Ser Phe Cys Asp Ser Tyr Pro Glu Leu Thr Gly Glu Asp Ile His Ser
325 330 335
Leu Asn Pro Glu Leu Glu Ser Ser Thr Ser Leu Asp Ser Asn Ser Ser

340 345 350

Gln Asp Leu Val Gly Gly Ala Val Pro Val Gln Ser His Ser Glu Asn

355 360 365

Phe Thr Ala Ala Thr Asp Leu Ser Arg Tyr Asn Asn Thr Leu Val Glu

370 375 380

Ser Ala Ser Thr Gln Asp Ala Leu Thr Met Arg Ser Gln Leu Asp Gln

385 390 395 400

Glu Ser Gly Ala Ile Ile His Pro Ala Thr Gln Thr Ser Leu Gln Glu

405 410 415

Ala

SEQ ID NO.: 2

Length: 1269 base pairs

Type: nucleic acid

Strandness: single

Topology: linear

Molecule type: cDNA to mRNA

Sequence

ATGGCTTTAA AAGTGCTACT AGAACAAGAG AAAACGTTTT TCACTCTTTT AGTATTACTA 60

GGCTATTTGT CATGTAAAGT GACTTGTGAA ACAGGAGACT GTAGACAGCA AGAATTCAGG 120

GATCGGTCTG GAAACTGTGT TCCCTGCAAC CAGTGTGGGC CAGGCATGGA GTTGTCTAAG 180

GAATGTGGCT TCGGCTATGG GGAGGATGCA CAGTGTGTGA CGTGCCGGCT GCACAGGTTC 240

AAGGAGGACT GGGGCTTCCA GAAATGCAAG CCCTGTCTGG ACTGCGCAGT GGTGAACCGC 300

TTTCAGAAGG CAAATTGTTC AGCCACCAGT GATGCCATCT GCGGGGACTG CTTGCCAGGA 360

TTTTATAGGA AGACGAAACT TGTCGGCTTT CAAGACATGG AGTGTGTGCC TTGTGGAGAC 420

CCTCCTCCTC CTTACGAACC GCACTGTGCC AGCAAGGTCA ACCTCGTGAA GATCGCGTCC 480

ACGGCCTCCA GCCCACGGGA CACGGCGCTG GCTGCCGTTA TCTGCAGCGC TCTGGCCACC 540

GTCCTGCTGG CCCTGCTCAT CCTCTGTGTC ATCTATTGTA AGAGACAGTT TATGGAGAAG 600

AAACCCAGCT GGTCTCTGCG GTCACAGGAC ATTCAGTACA ACGGCTCTGA GCTGTCGTGT 660

CTTGACAGAC CTCAGCTCCA CGAATATGCC CACAGAGCCT GCTGCCAGTG CCGCCGTGAC 720

TCAGTGCAGA CCTGCGGGCC GGTGCGCTTG CTCCCATCCA TGTGCTGTGA GGAGGCCTGC 780

AGCCCCAACC CGGCGACTCT TGGTTGTGGG GTGCATTCTG CAGCCAGTCT TCAGGCAAGA 840

AACGCAGGCC CAGCCGGGGA GATGGTGCCG ACTTTCTTCG GATCCCTCAC GCAGTCCATC 900

TGTGGCGAGT TTTCAGATGC CTGGCCTCTG ATGCAGAATC CCATGGGTGG TGACAACATC 960

TCTTTTTGTG ACTCTTATCC TGAACTCACT GGAGAAGACA TTCATTCTCT CAATCCAGAA 1020

CTTGAAAGCT CAACGTCTTT GGATTCAAAT AGCAGTCAAG ATTTGGTTGG TGGGGCTGTT 1080

CCAGTCCAGT CTCATTCTGA AAACTTTACA GCAGCTACTG ATTTATCTAG ATATAACAAC 1140

ACACTGGTAG AATCAGCATC AACTCAGGAT GCACTAACTA TGAGAAGCCA GCTAGATCAG 1200

GAGAGTGGCG CTATCATCCA CCCAGCCACT CAGACGTCCC TCCAGGTAAG GCAGCGACTG 1260

GGTTCCCTG 1269

SEQ ID NO.: 3

length: 1704 base pairs

Type: nucleic acid

Strandness: single

Topology: linear

Molecule type: cDNA to mRNA

Sequence

GGGAACGTAG AACTCTCCAA CAATAAATAC ATTTGATAAG AAAGATGGCT TTAAAAGTGC 60

TACTAGAACA AGAGAAAACG TTTTTCACTC TTTTAGTATT ACTAGGCTAT TTGTCATGTA 120

AAGTGACTTG TGAAACAGGA GACTGTAGAC AGCAAGAATT CAGGGATCGG TCTGGAAACT 180

GTGTTCCCTG CAACCAGTGT GGGCCAGGCA TGGAGTTGTC TAAGGAATGT GGCTTCGGCT 240

ATGGGGAGGA TGCACAGTGT GTGACGTGCC GGCTGCACAG GTTCAAGGAG GACTGGGGCT 300

TCCAGAAATG CAAGCCCTGT CTGGACTGCG CAGTGGTGAA CCGCTTTCAG AAGGCAAATT 360

GTTCAGCCAC CAGTGATGCC ATCTGCGGGG ACTGCTTGCC AGGATTTTAT AGGAAGACGA 420

AACTTGTCGG CTTTCAAGAC ATGGAGTGTG TGCCTTGTGG AGACCCTCCT CCTCCTTACG 480

AACCGCACTG TGCCAGCAAG GTCAACCTCG TGAAGATCGC GTCCACGGCC TCCAGCCCAC 540

GGGACACGGC GCTGGCTGCC GTTATCTGCA GCGCTCTGGC CACCGTCCTG CTGGCCCTGC 600

TCATCCTCTG TGTCATCTAT TGTAAGAGAC AGTTTATGGA GAAGAAACCC AGCTGGTCTC 660

TGCGGTCACA GGACATTCAG TACAACGGCT CTGAGCTGTC GTGTCTTGAC AGACCTCAGC 720

TCCACGAATA TGCCCACAGA GCCTGCTGCC AGTGCCGCCG TGACTCAGTG CAGACCTGCG 780

GGCCGGTGCG CTTGCTCCCA TCCATGTGCT GTGAGGAGGC CTGCAGCCCC AACCCGGCGA 840

CTCTTGGTTG TGGGGTGCAT TCTGCAGCCA GTCTTCAGGC AAGAAACGCA GGCCCAGCCG 900

GGGAGATGGT GCCGACTTTC TTCGGATCCC TCACGCAGTC CATCTGTGGC GAGTTTTCAG 960

ATGCCTGGCC TCTGATGCAG AATCCCATGG GTGGTGACAA CATCTCTTTT TGTGACTCTT 1020

ATCCTGAACT CACTGGAGAA GACATTCATT CTCTCAATCC AGAACTTGAA AGCTCAACGT 1080

CTTTGGATTC AAATAGCAGT CAAGATTTGG TTGGTGGGGC TGTTCCAGTC CAGTCTCATT 1140

CTGAAAACTT TACAGCAGCT ACTGATTTAT CTAGATATAA CAACACACTG GTAGAATCAG 1200

CATCAACTCA GGATGCACTA ACTATGAGAA GCCAGCTAGA TCAGGAGAGT GGCGCTATCA 1260

TCCACCCAGC CACTCAGACG TCCCTCCAGG AAGCTTAAAG AACCTGCTTC TTTCTGCAGT 1320

AGAAGCGTGT GCTGGAACCC AAAGAGTACT CCTTTGTTAG GCTTATGGAC TGAGCAGTCT 1380

GGACCTTGCA TGGCTTCTGG GGCAAAAATA AATCTGAACC AAACTGACGG CATTTGAAGC 1440

CTTTCAGCCA GTTGCTTCTG AGCCAGACCA GCTGTAAGCT GAAACCTCAA TGAATAACAA 1500

GAAAAGACTC CAGGCCGACT CATGATACTC TGCATCTTTC CTACATGAGA AGCTTCTCTG 1560

CCACAAAAGT GACTTCAAAG ACGGATGGGT TGAGCTGGCA GCCTATGAGA TTGTGGACAT 1620

ATAACAAGAA ACAGAAATGC CCTCATGCTT ATTTTCATGG TGATTGTGGT TTTACAAGAC 1680

TGAAGACCCA GAGTATACTT TTTC 1704

SEQ ID NO.: 4

Length: 1704 base pairs

Type: nucleic acid

Strandness: single

Topology: linear

Molecule type: cDNA to mRNA

Original source:

Organism: Homo Sapiens

Cell line: HAS303

Feature

Name/Key: CDS

Location: 45..1295

Identification method: P

Name/Key: sig peptide

Location: 45..119

Identification method: S

Name/Key: mat peptide

Location: 120..1295

Identification method: S

Sequecne

GGGAACGTAG AACTCTCCAA CAATAAATAC ATTTGATAAG AAAG ATG GCT TTA AAA 56
Met Ala Leu Lys
-25

GTG CTA CTA GAA CAA GAG AAA ACG TTT TTC ACT CTT TTA GTA TTA CTA 104
Val Leu Leu Glu Gln Glu Lys Thr Phe Phe Thr Leu Leu Val Leu Leu
-20 -15 -10

GGC TAT TTG TCA TGT AAA GTG ACT TGT GAA ACA GGA GAC TGT AGA CAG 152
Gly Tyr Leu Ser Cys Lys Val Thr Cys Glu Thr Gly Asp Cyc Arg Gln
-5 1 5 10

CAA GAA TTC AGG GAT CGG TCT GGA AAC TGT GTT CCC TGC AAC CAG TGT 200
Gln Glu Phe Arg Asp Arg Ser Gly Asn Cys Val Pro Cys Asn Gln Cys
15 20 25

GGG CCA GGC ATG GAG TTG TCT AAG GAA TGT GGC TTC GGC TAT GGG GAG 248
Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe Gly Tyr Gly Glu
30 35 40

GAT GCA CAG TGT GTG ACG TGC CGG CTG CAC AGG TTC AAG GAG GAC TGG 296
Asp Ala Gln Cys Val Thr Cys Arg Leu His Arg Phe Lys Glu Asp Trp
45 50 55

GGC TTC CAG AAA TGC AAG CCC TGT CTG GAC TGC GCA GTG GTG AAC CGC 344
Gly Phe Gln Lys Cys Lys Pro Cys Leu Asp Cys Ala Val Val Asn Arg
60 65 70 75

TTT CAG AAG GCA AAT TGT TCA GCC ACC AGT GAT GCC ATC TGC GGG GAC 392
Phe Gln Lys Ala Asn Cys Ser Ala Thr Ser Asp Ala Ile Cys Gly Asp
80 85 90

TGC TTG CCA GGA TTT TAT AGG AAG ACG AAA CTT GTC GGC TTT CAA GAC 440

Cys Leu Pro Gly Phe Tyr Arg Lys Thr Lys Leu Val Gly Phe Gln Asp

95 100 105

ATG GAG TGT GTG CCT TGT GGA GAC CCT CCT CCT CCT TAC GAA CCG CAC 488

Met Glu Cys Val Pro Cys Gly Asp Pro Pro Pro Pro Tyr Glu Pro His

110 115 120

TGT GCC AGC AAG GTC AAC CTC GTG AAG ATC GCG TCC ACG GCC TCC AGC 536

Cys Ala Ser Lys Val Asn Leu Val Lys Ile Ala Ser Thr Ala Ser Ser

125 130 135

CCA CGG GAC ACG GCG CTG GCT GCC GTT ATC TGC AGC GCT CTG GCC ACC 584

Pro Arg Asp Thr Ala Leu Ala Ala Val Ile Cys Ser Ala Leu Ala Thr

140 145 150 155

GTC CTG CTG GCC CTG CTC ATC CTC TGT GTC ATC TAT TGT AAG AGA CAG 632

Val Leu Leu Ala Leu Leu Ile Leu Cys Val Ile Tyr Cys Lys Arg Gln

160 165 170

TTT ATG GAG AAG AAA CCC AGC TGG TCT CTG CGG TCA CAG GAC ATT CAG 680

Phe Met Glu Lys Lys Pro Ser Trp Ser Leu Arg Ser Gln Asp Ile Gln

175 180 185

TAC AAC GGC TCT GAG CTG TCG TGT CTT GAC AGA CCT CAG CTC CAC GAA 728

Tyr Asn Gly Ser Glu Leu Ser Cys Leu Asp Rro Arg Gln Leu His Glu

190 195 200

TAT GCC CAC AGA GCC TGC TGC CAG TGC CGC CGT GAC TCA GTG CAG ACC 776

Tyr Ala His Arg Ala Cys Cys Gln Cys Arg Arg Asp Ser Val Gln Thr

205 210 215

TGC GGG CCG GTG CGC TTG CTC CCA TCC ATG TGC TGT GAG GAG GCC TGC 824

Cys Gly Pro Val Arg Leu Leu Pro Ser Met Cys Cys Glu Glu Ala Cys

220 225 230 235

AGC CCC AAC CCG GCG ACT CTT GGT TGT GGG GTG CAT TCT GCA GCC AGT 872

Ser Pro Asn Pro Ala Thr Leu Gly Cys Gly Val His Ser Ala Ala Ser

240 245 250

CTT CAG GCA AGA AAC GCA GGC CCA GCC GGG GAG ATG GTG CCG ACT TTC 920

Leu Gln Ala Arg Asn Ala Gly Pro Ala Gly Glu Met Val Pro Thr Phe

255 260 265

TTC GGA TCC CTC ACG CAG TCC ATC TGT GGC GAG TTT TCA GAT GCC TGG 968

Phe Gly Ser Leu Thr Gln Ser Ile Cys Gly Glu Phe Ser Asp Ala Trp

270 275 280

CCT CTG ATG CAG AAT CCC ATG GGT GGT GAC AAC ATC TCT TTT TGT GAC 1016

Pro Leu Met Gln Asn Pro Met Gly Gly Asp Asn Ile Ser Phe Cys Asp

285 290 295

TCT TAT CCT GAA CTC ACT GGA GAA GAC ATT CAT TCT CTC AAT CCA GAA 1064

Ser Tyr Pro Glu Leu Thr Gly Glu Asp Ile His Ser Leu Asn Pro Glu

300 305 310 315

CTT GAA AGC TCA ACG TCT TTG GAT TCA AAT AGC AGT CAA GAT TTG GTT 1112

Leu Glu Ser Ser Thr Ser Leu Asp Ser Asn Ser Ser Gln Asp Leu Val

320 325 330

GGT GGG GCT GTT CCA GTC CAG TCT CAT TCT GAA AAC TTT ACA GCA GCT 1160

Gly Gly Ala Val Pro Val Gln Ser His Ser Glu Asn Phe Thr Ala Ala

335 340 345

ACT GAT TTA TCT AGA TAT AAC AAC ACA CTG GTA GAA TCA GCA TCA ACT 1208

Thr Asp Leu Ser Arg Tyr Asn Asn Thr Leu Val Glu Ser Ala Ser Thr

350 355 360

CAG GAT GCA CTA ACT ATG AGA AGC CAG CTA GAT CAG GAG AGT GGC GCT 1256

Gln Asp Ala Leu Thr Met Arg Ser Gln Leu Asp Gln Glu Ser Gly Ala

365 370 375

ATC ATC CAC CCA GCC ACT CAG ACG TCC CTC CAG GAA GCT TAAAGAACCT 1305

Ile Ile His Pro Ala Thr Gln Thr Ser Leu Gln Glu Ala

380 385 390

GCTTCTTTCT GCAGTAGAAG CGTGTGCTGG AACCCAAAGA GTACTCCTTT GTTAGGCTTA 1365

TGGACTGAGC AGTCTGGACC TTGCATGGCT TCTGGGGCAA AAATAAATCT GAACCAAACT 1425

GACGGCATTT GAAGCCTTTC AGCCAGTTGC TTCTGAGCCA GACCAGCTGT AAGCTGAAAC 1485

CTCAATGAAT AACAAGAAAA GACTCCAGGC CGACTCATGA TACTCTGCAT CTTTCCTACA 1545

TGAGAAGCTT CTCTGCCACA AAAGTGACTT CAAAGACGGA TGGGTTGAGC TGGCAGCCTA 1605

TGAGATTGTG GACATATAAC AAGAAACAGA AATGCCCTCA TGCTTATTTT CATGGTGATT 1665

GTGGTTTTAC AAGACTGAAG ACCCAGAGTA TACTTTTTC 1704

SEQ ID NO.: 5

Length: 423 amino acids

Type: amino acid

Topology: linear

Molecule type: protein

Sequence

Met Ala Leu Lys Val Leu Leu Glu Gln Glu Lys Thr Phe Phe Thr Leu

1 5 10 15

Leu Val Leu Leu Gly Tyr Leu Ser Cys Lys Val Thr Cys Glu Thr Gly

20 25 30

Asp Cys Arg Gln Gln Glu Phe Arg Asp Arg Ser Gly Asn Cys Val Pro

35 40 45

Cys Asn Gln Cys Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe

50 55 60

Gly Tyr Gly Glu Asp Ala Gln Cys Val Thr Cys Arg Leu His Arg Phe

65 70 75 80

Lys Glu Asp Trp Gly Phe Gln Lys Cys Lys Pro Cys Leu Asp Cys Ala

85 90 95

Val Val Asn Arg Phe Gln Lys Ala Asn Cys Ser Ala Thr Ser Asp Ala

100 105 110

Ile Cys Gly Asp Cys Leu Pro Gly Phe Tyr Arg Lys Thr Lys Leu Val

115 120 125

Gly Phe Gln Asp Met Glu Cys Val Pro Cys Gly Asp Pro Pro Pro Pro

130 135 140

Tyr Glu Pro His Cys Ala Ser Lys Val Asn Leu Val Lys Ile Ala Ser

145 150 155 160

Thr Ala Ser Ser Pro Arg Asp Thr Ala Leu Ala Ala Val Ile Cys Ser

165 170 175

Ala Leu Ala Thr Val Leu Leu Ala Leu Leu Ile Leu Cys Val Ile Tyr

180 185 190

Cys Lys Arg Gln Phe Met Glu Lys Lys Pro Ser Trp Ser Leu Arg Ser

195 200 205

Gln Asp Ile Gln Tyr Asn Gly Ser Glu Leu Ser Cys Leu Asp Pro Arg

210 215 220

Gln Leu His Glu Tyr Ala His Arg Ala Cys Cys Gln Cys Arg Arg Asp

225 230 235 240

Ser Val Gln Thr Cys Gly Pro Val Arg Leu Leu Pro Ser Met Cys Cys

245 250 255

Glu Glu Ala Cys Ser Pro Asn Pro Ala Thr Leu Gly Cys Gly Val His

260 265 270

Ser Ala Ala Ser Leu Gln Ala Arg Asn Ala Gly Pro Ala Gly Glu Met

275 280 285

Val Pro Thr Phe Phe Gly Ser Leu Thr Gln Ser Ile Cys Gly Glu Phe

290 295 300

Ser Asp Ala Trp Pro Leu Met Gln Asn Pro Met Gly Gly Asp Asn Ile

305 310 315 320

Ser Phe Cys Asp Ser Tyr Pro Glu Leu Thr Gly Glu Asp Ile His Ser

325 330 335

Leu Asn Pro Glu Leu Glu Ser Ser Thr Ser Leu Asp Ser Asn Ser Ser

340 345 350

Gln Asp Leu Val Gly Gly Ala Val Pro Val Gln Ser His Ser Glu Asn

355 360 365

Phe Thr Ala Ala Thr Asp Leu Ser Arg Tyr Asn Asn Thr Leu Val Glu

370 375 380

Ser Ala Ser Thr Gln Asp Ala Leu Thr Met Arg Ser Gln Leu Asp Gln

385 390 395 400

Glu Ser Gly Ala Ile Ile His Pro Ala Thr Gln Thr Ser Leu Gln Val

405 410 415

Arg Gln Arg Leu Gly Ser Leu

420

SEQ ID NO.: 6

Length: 1269 base pairs

Type: nucleic acid

Strandness: single

Topology: linear

Molecule type: cDNA to mRNA

Sequecne

ATGGCTTTAA AAGTGCTACT AGAACAAGAG AAAACGTTTT TCACTCTTTT AGTATTACTA 60

GGCTATTTGT CATGTAAAGT GACTTGTGAA ACAGGAGACT GTAGACAGCA AGAATTCAGG 120

GATCGGTCTG GAAACTGTGT TCCCTGCAAC CAGTGTGGGC CAGGCATGGA GTTGTCTAAG 180

GAATGTGGCT TCGGCTATGG GGAGGATGCA CAGTGTGTGA CGTGCCGGCT GCACAGGTTC 240

AAGGAGGACT GGGGCTTCCA GAAATGCAAG CCCTGTCTGG ACTGCGCAGT GGTGAACCGC 300

TTTCAGAAGG CAAATTGTTC AGCCACCAGT GATGCCATCT GCGGGGACTG CTTGCCAGGA 360

TTTTATAGGA AGACGAAACT TGTCGGCTTT CAAGACATGG AGTGTGTGCC TTGTGGAGAC 420

CCTCCTCCTC CTTACGAACC GCACTGTGCC AGCAAGGTCA ACCTCGTGAA GATCGCGTCC 480

ACGGCCTCCA GCCCACGGGA CACGGCGCTG GCTGCCGTTA TCTGCAGCGC TCTGGCCACC 540

GTCCTGCTGG CCCTGCTCAT CCTCTGTGTC ATCTATTGTA AGAGACAGTT TATGGAGAAG 600

AAACCCAGCT GGTCTCTGCG GTCACAGGAC ATTCAGTACA ACGGCTCTGA GCTGTCGTGT 660

CTTGACAGAC CTCAGCTCCA CGAATATGCC CACAGAGCCT GCTGCCAGTG CCGCCGTGAC 720

TCAGTGCAGA CCTGCGGGCC GGTGCGCTTG CTCCCATCCA TGTGCTGTGA GGAGGCCTGC 780

AGCCCCAACC CGGCGACTCT TGGTTGTGGG GTGCATTCTG CAGCCAGTCT TCAGGCAAGA 840

AACGCAGGCC CAGCCGGGGA GATGGTGCCG ACTTTCTTCG GATCCCTCAC GCAGTCCATC 900

TGTGGCGAGT TTTCAGATGC CTGGCCTCTG ATGCAGAATC CCATGGGTGG TGACAACATC 960

TCTTTTTGTG ACTCTTATCC TGAACTCACT GGAGAAGACA TTCATTCTCT CAATCCAGAA 1020

CTTGAAAGCT CAACGTCTTT GGATTCAAAT AGCAGTCAAG ATTTGGTTGG TGGGGCTGTT 1080

CCAGTCCAGT CTCATTCTGA AAACTTTACA GCAGCTACTG ATTTATCTAG ATATAACAAC 1140

ACACTGGTAG AATCAGCATC AACTCAGGAT GCACTAACTA TGAGAAGCCA GCTAGATCAG 1200

GAGAGTGGCG CTATCATCCA CCCAGCCACT CAGACGTCCC TCCAGGTAAG GCAGCGACTG 1260

GGTTCCCTG 1269

SEQ ID NO.: 7

Length: 1496 base pairs

Type: nucleic acid

Strandness: single

Topology: linear

Molecule type: cDNA to mRNA

Sequence

GGGAACGTAG AACTCTCCAA CAATAAATAC ATTTGATAAG AAAGATGGCT TTAAAAGTGC 60

TACTAGAACA AGAGAAAACG TTTTTCACTC TTTTAGTATT ACTAGGCTAT TTGTCATGTA 120

AAGTGACTTG TGAAACAGGA GACTGTAGAC AGCAAGAATT CAGGGATCGG TCTGGAAACT 180

GTGTTCCCTG CAACCAGTGT GGGCCAGGCA TGGAGTTGTC TAAGGAATGT GGCTTCGGCT 240

ATGGGGAGGA TGCACAGTGT GTGACGTGCC GGCTGCACAG GTTCAAGGAG GACTGGGGCT 300

TCCAGAAATG CAAGCCCTGT CTGGACTGCG CAGTGGTGAA CCGCTTTCAG AAGGCAAATT 360

GTTCAGCCAC CAGTGATGCC ATCTGCGGGG ACTGCTTGCC AGGATTTTAT AGGAAGACGA 420

AACTTGTCGG CTTTCAAGAC ATGGAGTGTG TGCCTTGTGG AGACCCTCCT CCTCCTTACG 480

AACCGCACTG TGCCAGCAAG GTCAACCTCG TGAAGATCGC GTCCACGGCC TCCAGCCCAC 540

GGGACACGGC GCTGGCTGCC GTTATCTGCA GCGCTCTGGC CACCGTCCTG CTGGCCCTGC 600

TCATCCTCTG TGTCATCTAT TGTAAGAGAC AGTTTATGGA GAAGAAACCC AGCTGGTCTC 660

TGCGGTCACA GGACATTCAG TACAACGGCT CTGAGCTGTC GTGTCTTGAC AGACCTCAGC 720

TCCACGAATA TGCCCACAGA GCCTGCTGCC AGTGCCGCCG TGACTCAGTG CAGACCTGCG 780

GGCCGGTGCG CTTGCTCCCA TCCATGTGCT GTGAGGAGGC CTGCAGCCCC AACCCGGCGA 840

CTCTTGGTTG TGGGGTGCAT TCTGCAGCCA GTCTTCAGGC AAGAAACGCA GGCCCAGCCG 900

GGGAGATGGT GCCGACTTTC TTCGGATCCC TCACGCAGTC CATCTGTGGC GAGTTTTCAG 960

ATGCCTGGCC TCTGATGCAG AATCCCATGG GTGGTGACAA CATCTCTTTT TGTGACTCTT 1020

ATCCTGAACT CACTGGAGAA GACATTCATT CTCTCAATCC AGAACTTGAA AGCTCAACGT 1080

CTTTGGATTC AAATAGCAGT CAAGATTTGG TTGGTGGGGC TGTTCCAGTC CAGTCTCATT 1140

CTGAAAACTT TACAGCAGCT ACTGATTTAT CTAGATATAA CAACACACTG GTAGAATCAG 1200

CATCAACTCA GGATGCACTA ACTATGAGAA GCCAGCTAGA TCAGGAGAGT GGCGCTATCA 1260

TCCACCCAGC CACTCAGACG TCCCTCCAGG TAAGGCAGCG ACTGGGTTCC CTGTGAACAC 1320

AGCACTGACT TACAGTAGAT CAGAACTCTG TTCCCAGCAT AAGATTTGGG GGAACCTGAT 1380

GAGTTTTTTT TTTGCATCTT TAATAATTTC TTGTATGTTG TAGAGTATGT TTTAAAATAA 1440

ATTTCAAGTA TTTTTTTTAA AAACTAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAA 1496

SEQ ID NO.: 8

Length: 1496 base pairs

Type: nucleic acid

Strandness: single

Topology: linear

Molecule type: cDNA to mRNA

Original source

Organism: Homo Sapiens

Cell line: HAS303

Feature

Name/Key: CDS

Location: 45..1313

Identification method: P

Name/Key: sig peptide

Location: 45..119

Identification method: S

Name/Key: mat peptide

Location: 120..1313

Identification method: S

Sequence

GGGAACGTAG AACTCTCCAA CAATAAATAC ATTTGATAAG AAAG ATG GCT TTA AAA 56

Met Ala Leu Lys

-25

GTG CTA CTA GAA CAA GAG AAA ACG TTT TTC ACT CTT TTA GTA TTA CTA 104

Val Leu Leu Glu Gln Glu Lys Thr Phe Phe Thr Leu Leu Val Leu Leu

-20 -15 -10

GGC TAT TTG TCA TGT AAA GTG ACT TGT GAA ACA GGA GAC TGT AGA CAG 152

Gly Tyr Leu Ser Cys Lys Val Thr Cys Glu Thr Gly Asp Cyc Arg Gln

-5 1 5 10

CAA GAA TTC AGG GAT CGG TCT GGA AAC TGT GTT CCC TGC AAC CAG TGT 200

Gln Glu Phe Arg Asp Arg Ser Gly Asn Cys Val Pro Cys Asn Gln Cys

15 20 25

GGG CCA GGC ATG GAG TTG TCT AAG GAA TGT GGC TTC GGC TAT GGG GAG 248

Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe Gly Tyr Gly Glu

30 35 40

GAT GCA CAG TGT GTG ACG TGC CGG CTG CAC AGG TTC AAG GAG GAC TGG 296

Asp Ala Gln Cys Val Thr Cys Arg Leu His Arg Phe Lys Glu Asp Trp

45 50 55

GGC TTC CAG AAA TGC AAG CCC TGT CTG GAC TGC GCA GTG GTG AAC CGC 344

Gly Phe Gln Lys Cys Lys Pro Cys Leu Asp Cys Ala Val Val Asn Arg

60 65 70 75

TTT CAG AAG GCA AAT TGT TCA GCC ACC AGT GAT GCC ATC TGC GGG GAC 392

Phe Gln Lys Ala Asn Cys Ser Ala Thr Ser Asp Ala Ile Cys Gly Asp

80 85 90

TGC TTG CCA GGA TTT TAT AGG AAG ACG AAA CTT GTC GGC TTT CAA GAC 440

Cys Leu Pro Gly Phe Tyr Arg Lys Thr Lys Leu Val Gly Phe Gln Asp

95 100 105

ATG GAG TGT GTG CCT TGT GGA GAC CCT CCT CCT CCT TAC GAA CCG CAC 488

Met Glu Cys Val Pro Cys Gly Asp Pro Pro Pro Pro Tyr Glu Pro His

110 115 120

TGT GCC AGC AAG GTC AAC CTC GTG AAG ATC GCG TCC ACG GCC TCC AGC 536

Cys Ala Ser Lys Val Asn Leu Val Lys Ile Ala Ser Thr Ala Ser Ser

125 130 135

CCA CGG GAC ACG GCG CTG GCT GCC GTT ATC TGC AGC GCT CTG GCC ACC 584

Pro Arg Asp Thr Ala Leu Ala Ala Val Ile Cys Ser Ala Leu Ala Thr

140 145 150 155

GTC CTG CTG GCC CTG CTC ATC CTC TGT GTC ATC TAT TGT AAG AGA CAG 632

Val Leu Leu Ala Leu Leu Ile Leu Cys Val Ile Tyr Cys Lys Arg Gln

160 165 170

TTT ATG GAG AAG AAA CCC AGC TGG TCT CTG CGG TCA CAG GAC ATT CAG 680

Phe Met Glu Lys Lys Pro Ser Trp Ser Leu Arg Ser Gln Asp Ile Gln

175 180 185

TAC AAC GGC TCT GAG CTG TCG TGT CTT GAC AGA CCT CAG CTC CAC GAA 728

Tyr Asn Gly Ser Glu Leu Ser Cys Leu Asp Rro Arg Gln Leu His Glu

190 195 200

TAT GCC CAC AGA GCC TGC TGC CAG TGC CGC CGT GAC TCA GTG CAG ACC 776

Tyr Ala His Arg Ala Cys Cys Gln Cys Arg Arg Asp Ser Val Gln Thr

205 210 215

TGC GGG CCG GTG CGC TTG CTC CCA TCC ATG TGC TGT GAG GAG GCC TGC 824

Cys Gly Pro Val Arg Leu Leu Pro Ser Met Cys Cys Glu Glu Ala Cys

220 225 230 235

AGC CCC AAC CCG GCG ACT CTT GGT TGT GGG GTG CAT TCT GCA GCC AGT 872

Ser Pro Asn Pro Ala Thr Leu Gly Cys Gly Val His Ser Ala Ala Ser

240 245 250

CTT CAG GCA AGA AAC GCA GGC CCA GCC GGG GAG ATG GTG CCG ACT TTC 920

Leu Gln Ala Arg Asn Ala Gly Pro Ala Gly Glu Met Val Pro Thr Phe

255 260 265

TTC GGA TCC CTC ACG CAG TCC ATC TGT GGC GAG TTT TCA GAT GCC TGG 968

Phe Gly Ser Leu Thr Gln Ser Ile Cys Gly Glu Phe Ser Asp Ala Trp

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          270                 275                 280
CCT CTG ATG CAG AAT CCC ATG GGT GGT GAC AAC ATC TCT TTT TGT GAC   1016
Pro Leu Met Gln Asn Pro Met Gly Gly Asp Asn Ile Ser Phe Cys Asp
    285                 290                 295
TCT TAT CCT GAA CTC ACT GGA GAA GAC ATT CAT TCT CTC AAT CCA GAA   1064
Ser Tyr Pro Glu Leu Thr Gly Glu Asp Ile His Ser Leu Asn Pro Glu
300                 305                 310                 315
CTT GAA AGC TCA ACG TCT TTG GAT TCA AAT AGC AGT CAA GAT TTG GTT   1112
Leu Glu Ser Ser Thr Ser Leu Asp Ser Asn Ser Ser Gln Asp Leu Val
                320                 325                 330
GGT GGG GCT GTT CCA GTC CAG TCT CAT TCT GAA AAC TTT ACA GCA GCT   1160
Gly Gly Ala Val Pro Val Gln Ser His Ser Glu Asn Phe Thr Ala Ala
            335                 340                 345
ACT GAT TTA TCT AGA TAT AAC AAC ACA CTG GTA GAA TCA GCA TCA ACT   1208
Thr Asp Leu Ser Arg Tyr Asn Asn Thr Leu Val Glu Ser Ala Ser Thr
        350                 355                 360
CAG GAT GCA CTA ACT ATG AGA AGC CAG CTA GAT CAG GAG AGT GGC GCT   1256
Gln Asp Ala Leu Thr Met Arg Ser Gln Leu Asp Gln Glu Ser Gly Ala
    365                 370                 375
ATC ATC CAC CCA GCC ACT CAG ACG TCC CTC CAG GTA AGG CAG CGA CTG   1304
Ile Ile His Pro Ala Thr Gln Thr Ser Leu Gln Val Arg Gln Arg Leu
380                 385                 390                 395
GGT TCC CTG TGAACACAG CACTGACTTA CAGTAGATCA GAACTCTGTT CCCAGCATAA 1362
Gly Ser Leu
GATTTGGGGG AACCTGATGA GTTTTTTTTT TGCATCTTTA ATAATTTCTT GTATGTTGTA 1422
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GAGTATGTTT TAAAATAAAT TTCAAGTATT TTTTTTAAAA ACTAAAAAAA AAAAAAAAAA 1482

AAAAAAAAAA AAAA 1496

SEQ ID NO.: 9

Length: 35 base pairs

Type: nucleoic acid

Strandness: single

Topology: linear

Sequence

CGATTGAATT CTAGACCTGC CTCGAGNNNN NNNNN

SEQ ID NO.: 10

Length: 28 base pairs

Type: nucleic acid

Strandness: single

Topology: linear

Sequence

AGAAAGATGG CTTTAAAAGT GCTACTAG

Claims:

1. Substantially purified form of the polypeptide that comprising the amino-acid sequence shown in SEQ ID NO. 1 or 5, homologue thereof, fragment thereof or homologue of the fragment.

2. A polypeptide according to claim 1 that comprising the amino-acid sequence shown in SEQ ID NO. 1 or 5.

3. A cDNA encoding the polypeptide according to claim 1.

4. A cDNA according to claim 3 that comprising the nucleotide sequence shown in SEQ ID NO. 2 or 6 or a fragment cDNA selectively hybridized to the cDNA.

5. A cDNA according to claim 3 that comprising the nucleotide sequence shown in SEQ ID NO. 3 or 8 or a fragment cDNA selectively hybridized to the cDNA.

6. A replication or expression vector carrying the cDNA according to claim 3 to 5.

7. A host cell transformed with the replication or expression vector according to claim 6.

8. A method for producing the polypeptide according to claim 1 or 2 which comprises culturing a host cell according to claim 7 under a condition effective to express the polypeptide according to claim 1 or 2.

9. A monoclonal or polyclonal antibody against the polypeptide according to claim 1 or 2.

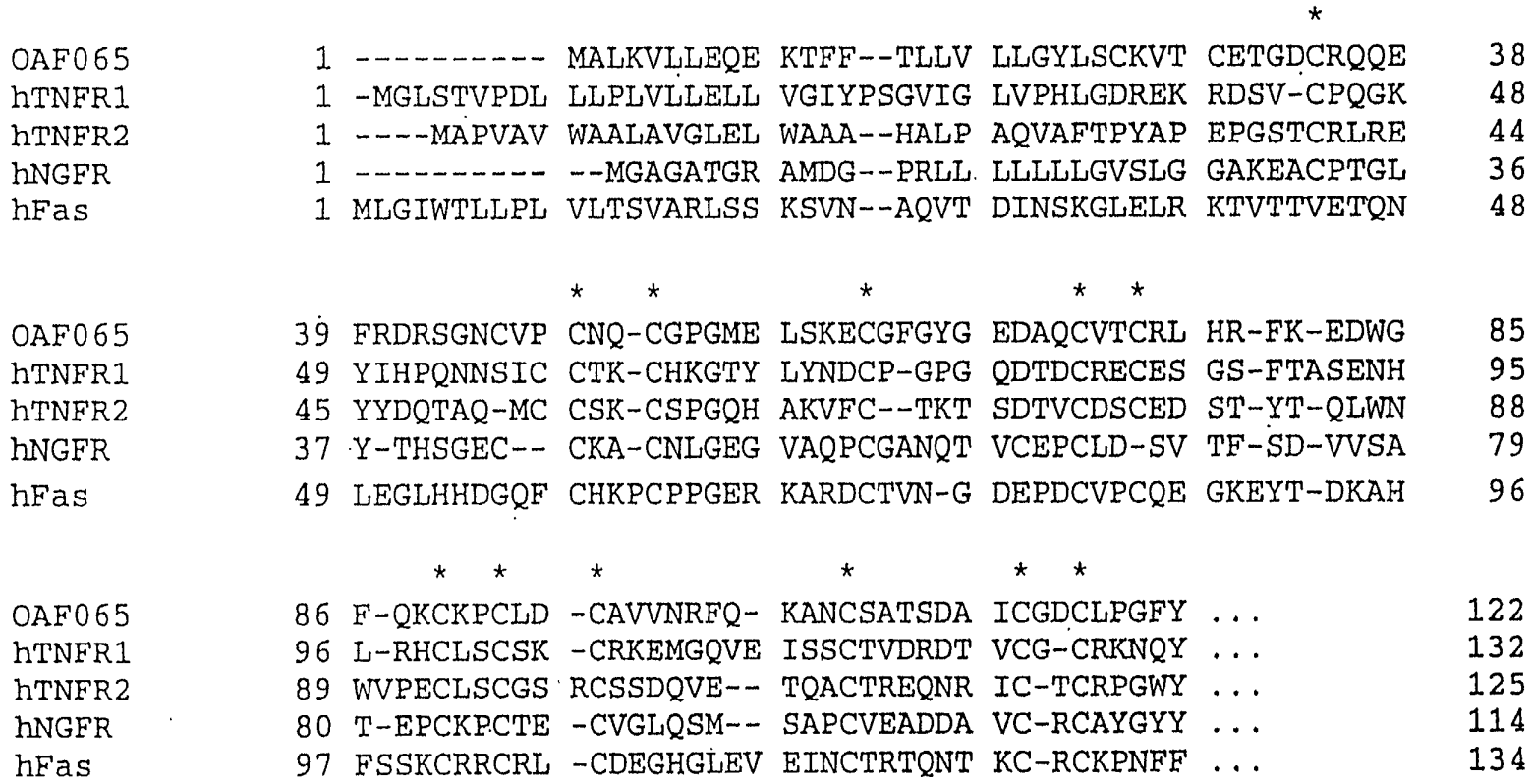
10. A pharmaceutical composition containing the

polypeptide according to claim 1 or 2 or the antibody according to claim 9, in association with pharmaceutically acceptable diluent and/or carrier.

Abstract

Polypeptide produced from human stromal cell line, the process for the preparation of the polypeptide, DNA encoding the polypeptide, vector carrying the DNA, host cell transformed by the vector, antibody of the polypeptide, and pharmaceutical composition containing the polypeptide or the antibody.

Fig. 1


OAF065 1 ---------- MALKVLLEQE KTFF--TLLV LLGYLSCKVT CETGDCRQQE 38
hTNFR1 1 -MGLSTVPDL LLPLVLLELL VGIYPSGVIG LVPHLGDREK RDSV-CPQGK 48
hTNFR2 1 ----MAPVAV WAALAVGLEL WAAA--HALP AQVAFTPYAP EPGSTCRLRE 44
hNGFR 1 ---------- --MGAGATGR AMDG--PRLL LLLLLGVSLG GAKEACPTGL 36
hFas 1 MLGIWTLLPL VLTSVARLSS KSVN--AQVT DINSKGLELR KTVTTVETQN 48
OAF065 39 FRDRSGNCVP CNQ-CGPGME LSKECGFGYG EDAQCVTCRL HR-FK-EDWG 85
hTNFR1 49 YIHPQNNSIC CTK-CHKGTY LYNDCP-GPG QDTDCRECES GS-FTASENH 95
hTNFR2 45 YYDQTAQ-MC CSK-CSPGQH AKVFC--TKT SDTVCDSCED ST-YT-QLWN 88
hNGFR 37 Y-THSGEC-- CKA-CNLGEG VAQPCGANQT VCEPCLD-SV TF-SD-VVSA 79
hFas 49 LEGLHHDGQF CHKPCPPGER KARDCTVN-G DEPDCVPCQE GKEYT-DKAH 96
OAF065 86 F-QKCKPCLD -CAVVNRFQ- KANCSATSDA ICGDCLPGFY ... 122
hTNFR1 96 L-RHCLSCSK -CRKEMGQVE ISSCTVDRDT VCG-CRKNQY ... 132
hTNFR2 89 WVPECLSCGS RCSSDQVE-- TQACTREQNR IC-TCRPGWY ... 125
hNGFR 80 T-EPCKPCTE -CVGLQSM-- SAPCVEADDA VC-RCAYGYY ... 114
hFas 97 FSSKCRRCRL -CDEGHGLEV EINCTRTQNT KC-RCKPNFF ... 134

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Novel polypeptides, DNAs encoding the polypeptides, and Utility of the polypeptides

the specification of which is attached hereto unless the following box is checked:

☒ was filed on February 26, 1998 as United States Application Number or PCT International Application Number PCT/JP98/00799 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information of which is material to the patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s) (Number)	(Country)	(Day/Month/Year Filed)	Priority Not Claimed
P. Hei. 9-43143	Japan	27/February/1997	☐
			☐
			☐

I hereby claim the benefits under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)

I hereby claim the benefits under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(Application Number)	(Filing Date)	(Status – patented, pending, abandoned)

I hereby appoint John H. Mion, Reg. No. 18,879; Thomas J. Macpeak, Reg. No. 19,292; Robert J. Seas, Jr., Reg. No. 21,092; Darryl Mexic, Reg. No. 23,063; Robert V. Sloan, Reg. No. 22,775; Peter D. Olexy, Reg. No. 24,513; J. Frank Osha, Reg. No. 24,625; Waddell A. Biggart, Reg. No. 24,861; Louis Gubinsky, Reg. No. 24,835; Neil B. Siegel, Reg. No. 25,200; David J. Cushing, Reg. No. 28,703; John R. Inge, Reg. No. 26,916; Joseph J. Ruch, Jr., Reg. No. 26,577; Sheldon I. Landsman, Reg. No. 25,430; Richard C. Turner, Reg. No. 29,710; Howard L. Bernstein, Reg. No. 25,665; Alan J. Kasper, Reg. No. 25,426; Kenneth J. Burchfiel, Reg. No. 31,333; Gordon Kit, Reg. No. 30,764; Susan J. Mack, Reg. No. 30,951; Frank L. Bernstein, Reg. No. 31,484; Mark Boland, Reg. No. 32,197; William H. Mandir, Reg. No. 32,156; Scott M. Daniels, Reg. No. 32,562, Brian W. Hannon, Reg. No. 32,778, Abraham J. Rosner, Reg. No. 33,276; Bruce E. Kramer, Reg. No. 33,725; Paul F. Neils, Reg. No. 33,102, Brett S. Sylvester, Reg. No. 32,765 and Robert M. Masters, Reg. No. 35,603; my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and request that all correspondence about the application be addressed to SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC, 2100 Pennsylvania Avenue, N.W., Washington, D.C. 20037.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Residence _____ Signature _____

_____ Post Office Address _____

Citizenship _____ _____

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Residence _____ Signature _____

_____ Post Office Address _____

Citizenship _____ _____

Date _____ Sixth Inventor _____
First Name Last Name

Residence _____ Signature _____

_____ Post Office Address _____

Citizenship _____ _____

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Citizenship _____ _____

(Supply similar information for ninth and subsequent joint inventors.)